

FIGURES

Fig. 1.

A

TatA (Eco)	M- SGISINQLEHTAVIVVLEFGTKKLG -----	26
TatE (Eco)	M- GEISITKLLVVAALVWLEFGTKKLR -----	26
TatAy (Bsu)	M- PTGPGSLAVIAIAVALTLEGPCKLP -----	25
TatAd (Bsu)	MFSNIGIPGLILIFVIAITTFGPKLP-----	27
TatAc (Bsu)	M- ELSFYKLLVLLFVGLFVGPDKLP -----	25
TatB (Eco)	M- DIGESELLVFLIGLVVLGPQRLP VAVKTVAGWIRALRSLATTVQNELTQELKLG	49
	* *	
TatA (Eco)	-----SIGSDLGASIKGFKKAMSDE-----PKQDKTSQDADFTAKTI	64
TatE (Eco)	-----TLGGDLGAAIKGFKKAMNDD-----A-AAKKGADVLDQAEKL	63
TatAy (Bsu)	-----ELGKAAGDTLREFKNATKGLT-----SDEEEKKKEDQ-----	57
TatAd (Bsu)	-----EIGRAAKRTLLEPKSATKSLV-----SGDEKEEKSAELTAVK-	64
TatAc (Bsu)	-----ALGRAAGKALSEFKQATSGLT-----QDIRKNDSN-----K-	57
TatB (Eco)	EFQDSLKKVEKASLTNLTPELKASMDLRLQAESMKRSYVANDPEKASDEAHTIHN	114
 *	
TatA (Eco)	ADKQADTNQE-----QAKTEDAKRHDKEQV	89
TatE (Eco)	SHKE-----	67
TatAy (Bsu)	-----	57
TatAd (Bsu)	-----QDKNAG	70
TatAc (Bsu)	-----EDKQM-	62
TatB (Eco)	VVKDNEAAHEGVTPAAAOQTQASSPEQKPEPTTPEPVVKPAADAEPKTAAPSPSSSDKP	171

B

TatC (Eco)	MSVEDTQ--PLITHLIELRKRL INCLIAVIVIFLCVVFANDIYH -LVSAPLIK	51
TatCy (Bsu)	MTRMKVNQMSLLEHIAELRKRL LIIVLAFVVFELAGFFLAKPIIVYLQETDEAK	50
TatCd (Bsu)	MDKKETH---LIGHLEELRRRL LIIVLAAAFELRLTAELFVQDIYDWLIRDLDGK	51
	*. . . . * * *	
TatC (Eco)	QLPQGSTMIA TDVASPFPT PKLTFMVSLHLSAEVLLYQVNAFIAPALYKHERR	105
TatCy (Bsu)	QL----TLNAFNLTDP LYVFMQFAFLICIVETSPVLLYQLWAFVSPGLYEKERR	104
TatCd (Bsu)	-----LAVLGPSEL LWVVMMLSGICATAASTPVAAYQLWRFVAPALTKTERK	98
 ** * *	
TatC (Eco)	LVVPLLIV---SSSL LYIGMAFAYFVVEPLARGEANTAPE -GVQVSTD LSYI	155
TatCy (Bsu)	VTLSYI---PVST LEFLAGLSPSYVTLFPPVDFMKRISQDLNVNQVIGINEYF	155
TatCd (Bsu)	VTIMYIMYIP GLEHLELAGISFCYEVLPVLSFLTHLSSG -HFETMFTADRYF	151
 * *	
TatC (Eco)	SFVMAIFMAFGVSEFVPVAIVLLCWMGITSPEDLRKKRPVIVGAKVVGMLTE	209
TatCy (Bsu)	HFLIQLTIPFGLLTQMPVILMPETRLGIVTPMFLAKIRKMAFETGLVTAALTE	209
TatCd (Bsu)	RFMNLSLPFCLEFELVLMFLTRLGILNPYRLAKAKLSYELGWSHLEP	205
	* * * *	
TatC (Eco)	PDVFSQTLAIPMYCLFEIGVFFSRF - VYKGRNREEENDAAEASEKTEE	258
TatCy (Bsu)	PELLSHMMVTVPLLDIYETSLISKAYRKAQKSSAADRDVSSG -----Q	254
TatCd (Bsu)	PDRISEFLVMEPLMLFEYSWLSAFVYKKRMRE -----ETAAA-----A	245
	* * *	

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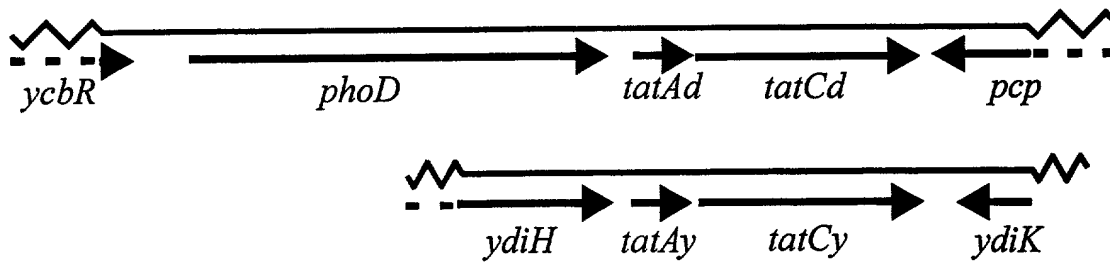
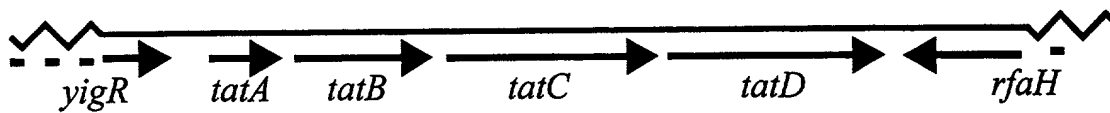
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Fig. 2.

A *B. subtilis*B *E. coli*

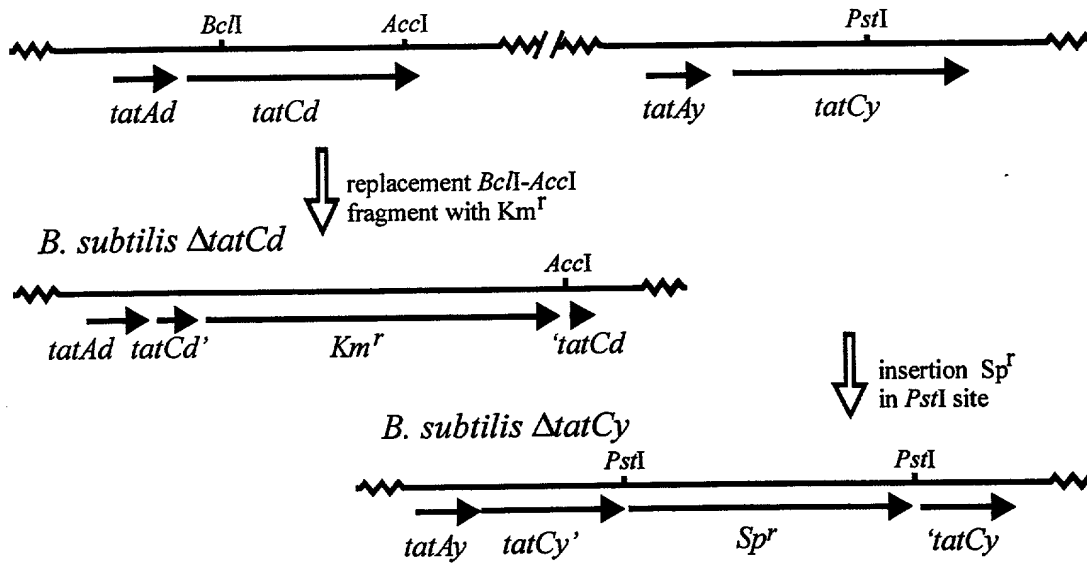
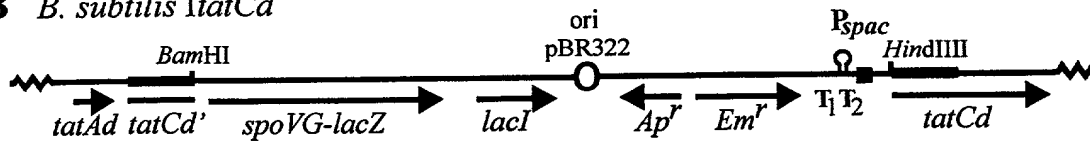
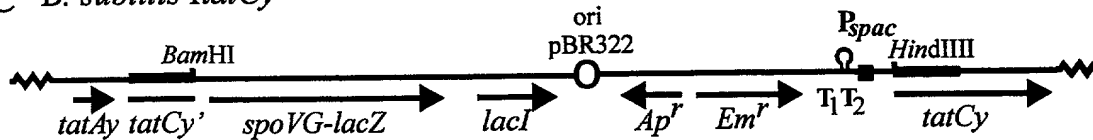
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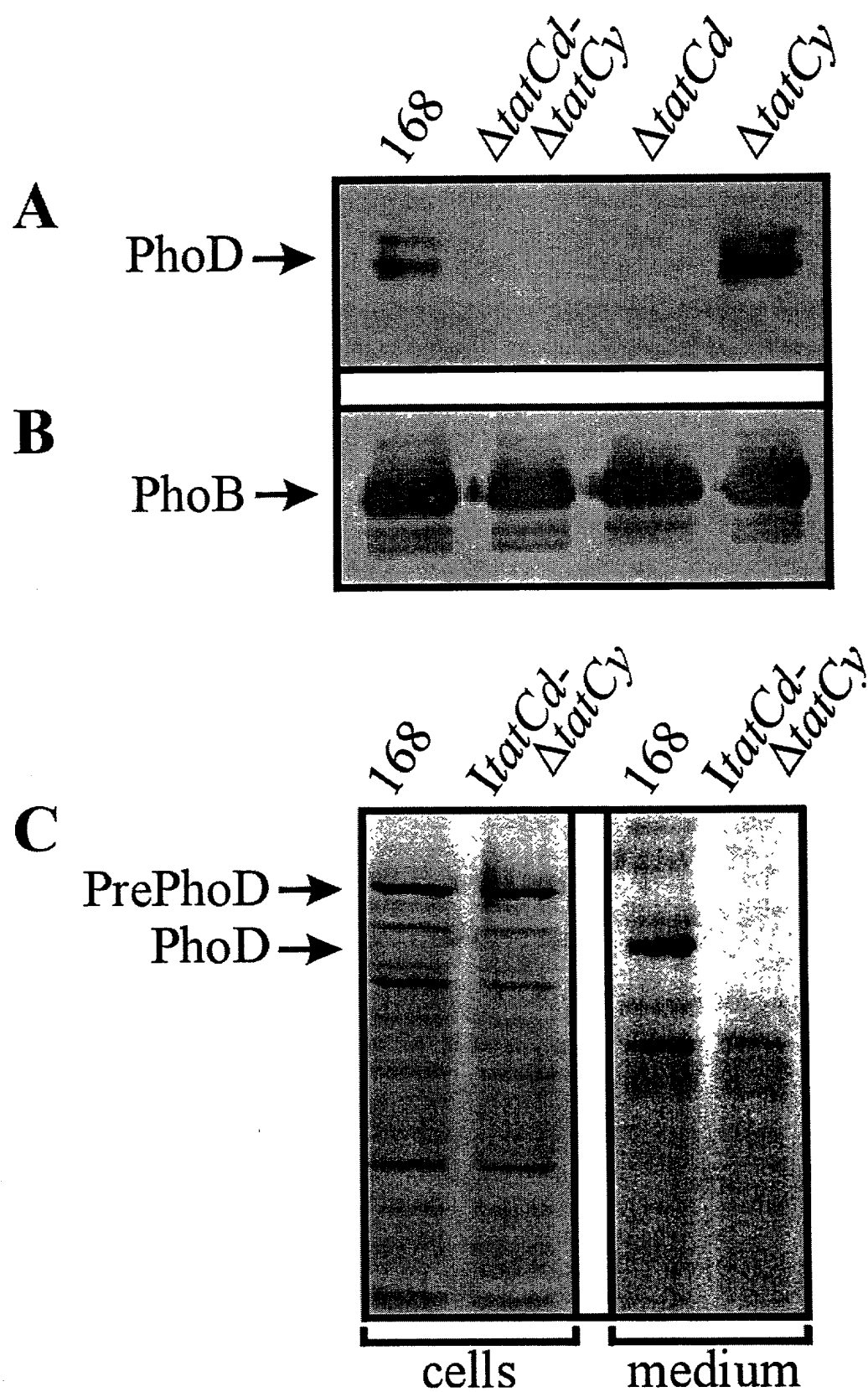
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Fig. 3.

A *B. subtilis* 168B *B. subtilis* 1*tatCd*C *B. subtilis* 1*tatCy*

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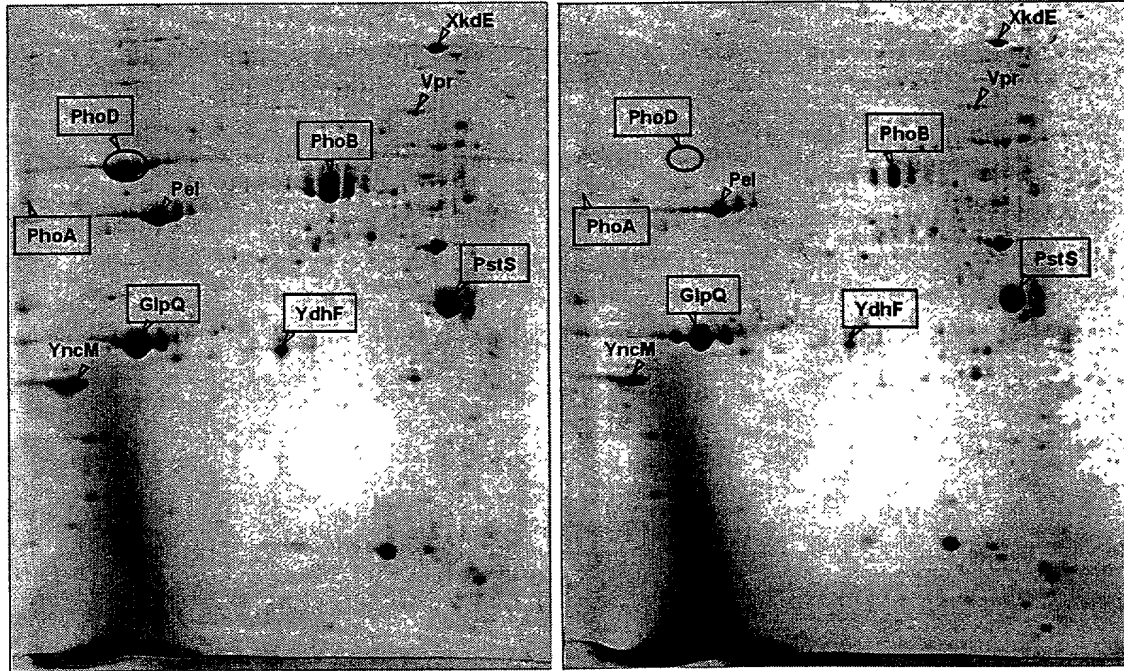
Fig. 4.



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Fig. 5.

168

 $\Delta tatCd-\Delta tatCy$ 

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FIGURE 6



Tat-dependent secretion of the *B. subtilis* lipase LipA. *B. subtilis* 168 (parental strain), *B. subtilis* ΔtatCd, *B. subtilis* ΔtatCy, or *B. subtilis* ΔtatCd-ΔtatCy were grown in TY-medium to end-exponential growth phase. To study the secretion of LipA, *B. subtilis* cells were separated from the growth medium by centrifugation. Proteins in the growth medium were concentrated 20-fold upon precipitation with trichloroacetic acid, and samples for polyacrylamide gel electrophoresis (SDS-PAGE) were prepared. Secreted LipA in the growth medium was visualized by SDS-PAGE and Western blotting, using LipA-specific antibodies.

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FIGURE 7

Predicted twin-arginine (RR-)signal peptides of *B. subtilis*¹

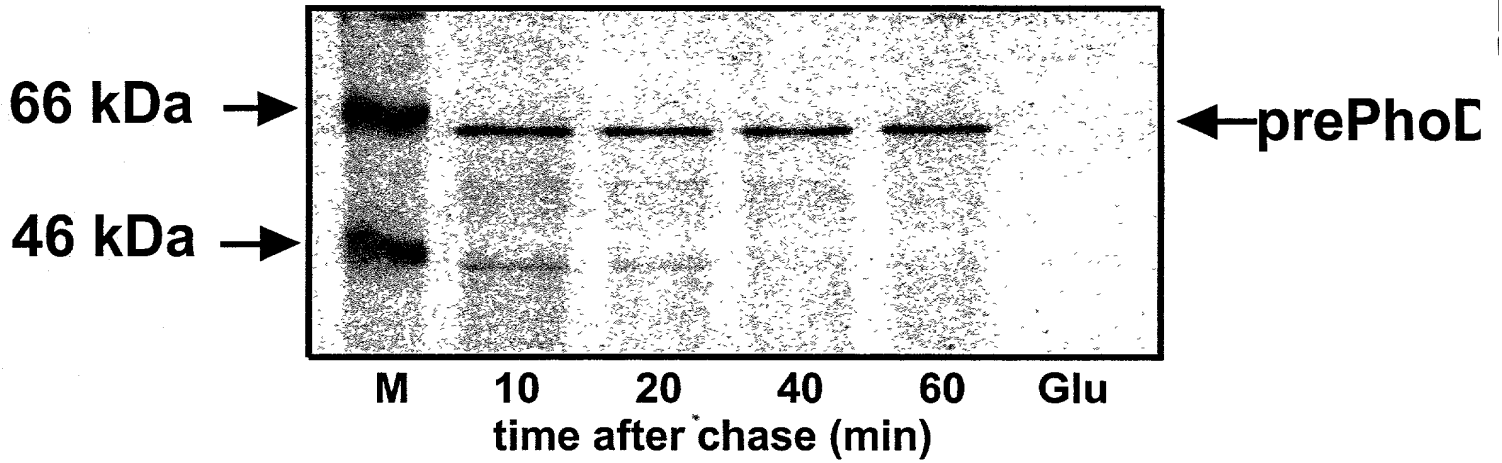
Protein	N	h	RR-Motif	H	h	C
AlbB	1	0.1	RRILL	27	2.0	AIA
AmyX TM	9	-0.8	RRSFE	15	1.1	-
AppB TM	8	0.5	RRTLm	19	2.3	-
LipA	7	-1.1	RRIIA	19	1.2	AKA
OppB TM	8	-0.6	RRLVY	24	2.0	-
PbpX	2	-2.2	RRRKL	14	2.9	WNA
PhoD	3	-1.3	RRKFI	17	0.9	VGA
QcrA TM	1	-1.1	RRQFL	19	1.3	-
TlpA TM	1	-0.8	RRLII	21	2.4	-
WapA ^W	1	-3.0	RRNFK	18	2.3	VLA
WprA	8	-1.7	RRKFS	20	1.9	AAA
YceA TM	1	-0.4	RRAFI	21	2.2	-
YesM TM	1	-1.5	RRMKI	20	2.4	QYA
YesW	1	-1.3	RRSCL	19	2.0	VKA
YfkN TM	1	-1.2	RRTHV	17	1.7	IHA
YkpC	8	-1.0	RRVAI	17	2.3	SLA
YkuE	1	-1.3	RRQFL	17	1.0	GYA
YmaC	7	0.0	RRFLL	15	2.4	YSL
YubF TM	9	-2.7	RRNTV	23	2.0	-
YuiC	8	0.2	RLLLM	20	1.9	IEA
YvhJ TM	2	-1.7	RRKIL	18	2.5	-
YwbN	1	-1.8	RRDIL	23	1.4	QTA

¹ The listed signal peptides contain, in addition to the twin-arginines, at least one other residue of the consensus sequence (R-R-X- ϕ - ϕ ; printed in bold). The number of residues in the N- and H-domains of each signal peptide, and the average hydrophobicity (h) of each of these domains, as determined by the algorithms of Kyte and Doolittle (Kyte, J., and R. F. Doolittle [1982] A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157:105-32), are indicated. Furthermore, the RR-motifs in the N-domain, and SPase I recognition sites in the C-domain (*ie.* positions -3 to -1 relative to the predicted SPase cleavage site) are shown. Proteins lacking a (putative) SPase I cleavage site, some of which contain additional transmembrane domains, are indicated with "TM". One protein containing cell wall binding repeats is indicated with "W".

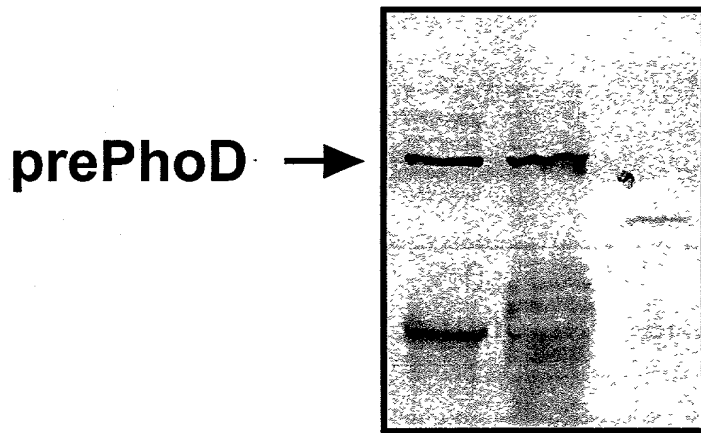
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A



B

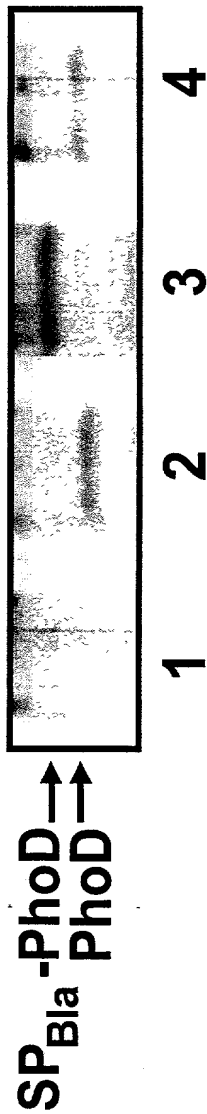


Proteinase K	-	+	+
Triton X-100	-	-	+

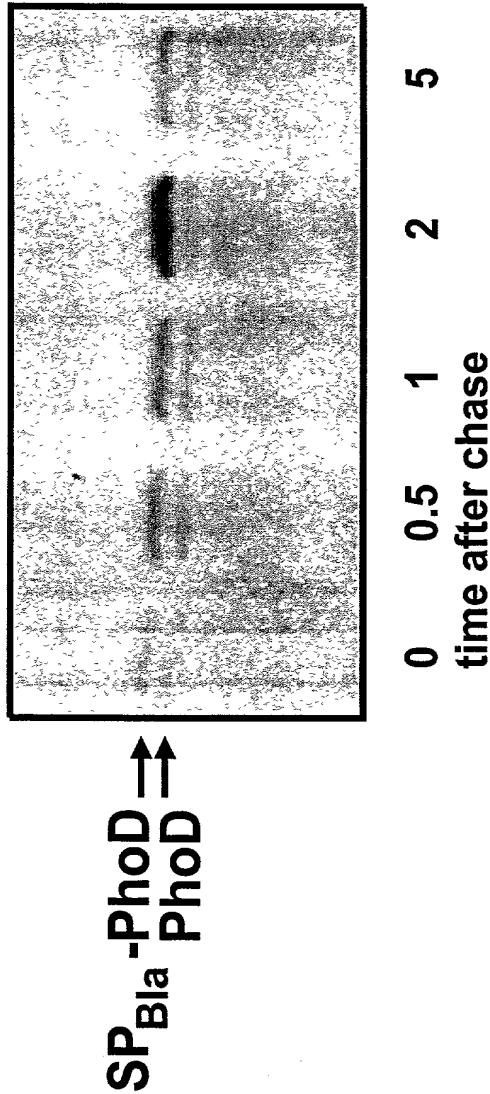
Figure **8**

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A



B untreated



C + NaN_3

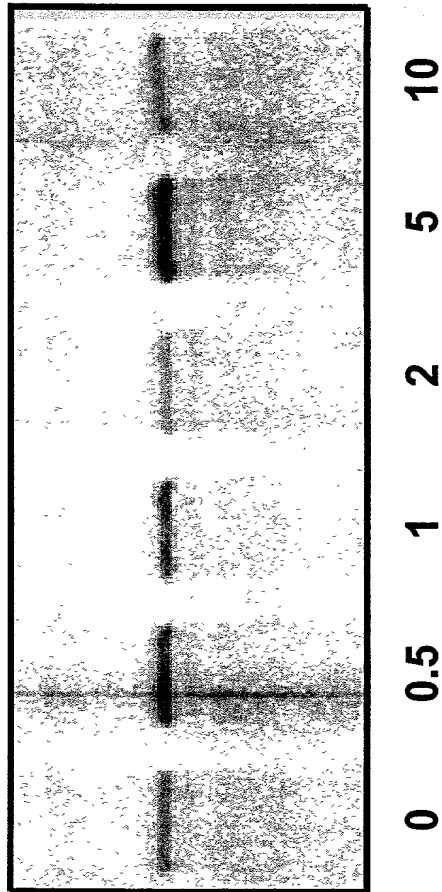
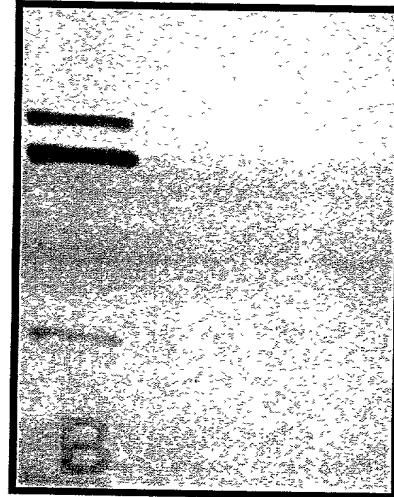
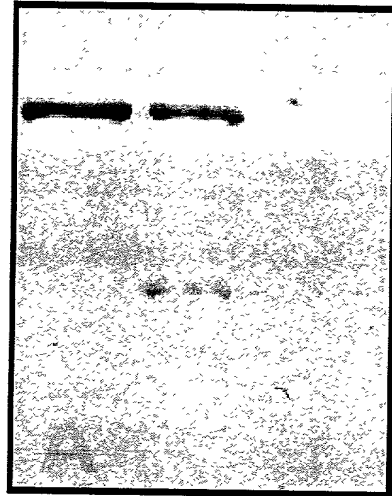


Figure 9

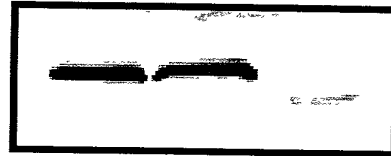
A

B

SP_{PhoD}-LacZ
LacZ



SecB



Proteinase K
Triton X-100

-	+	+
-	-	+

-	+	+
-	-	+

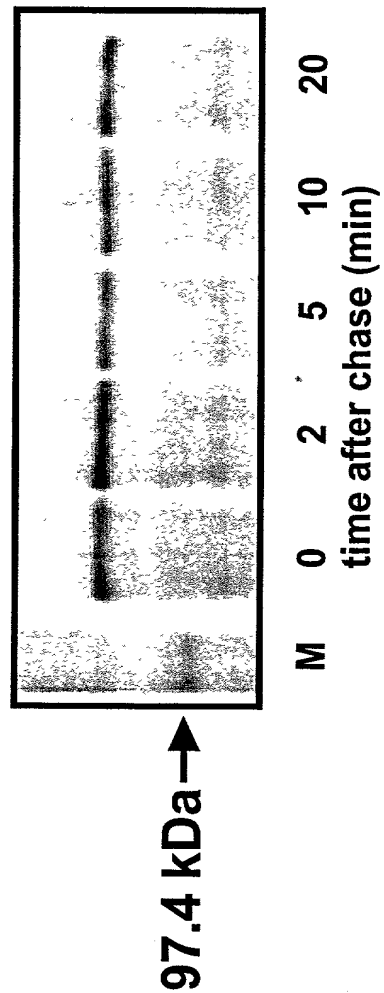
Figure 10

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TOCT60" 2E213550

A



B

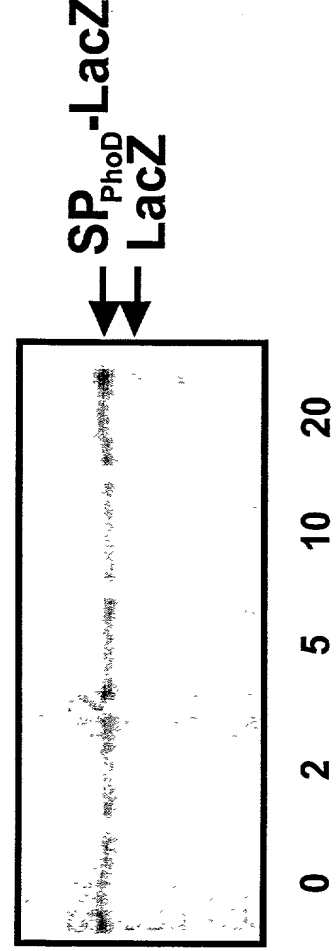
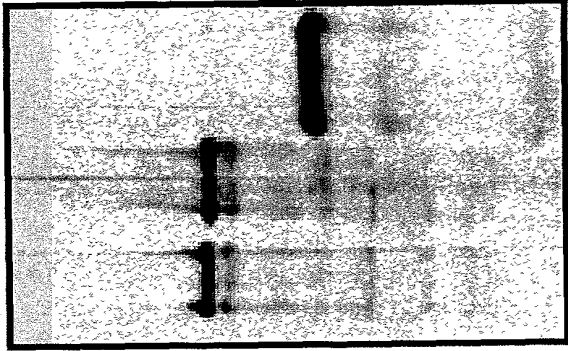


Figure 11

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A
+nigericin



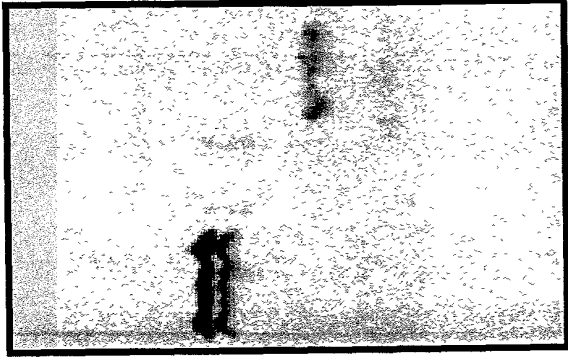
SP_{PhoD}-LacZ
LacZ

SecB

Proteinase K
Triton X-100

- + +
- - +

B
+NaN₃

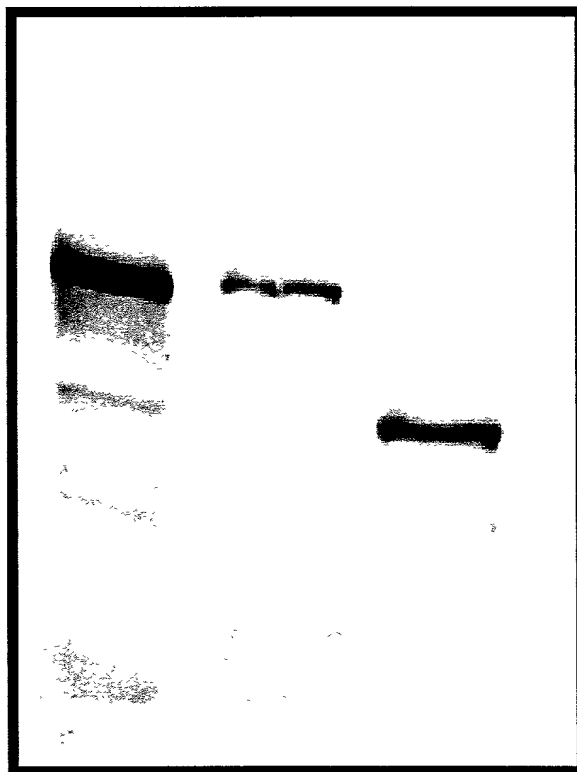


- + +
- - +

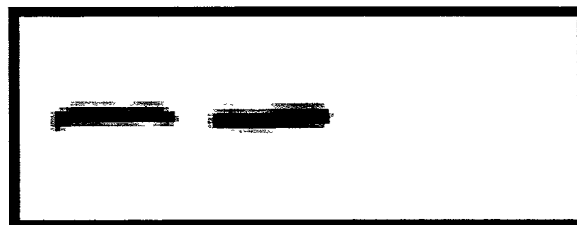
Figure 12

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SP_{PhoD}-LacZ →



SecB →



Proteinase K
Triton X-100

-	+	+
-	-	+

Figure 13

Figure 14
Homologs in *B. alcalophilus*

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TatA

**MGGLSVGSVVLIALVALLIFGPKKLPELGKAAGSTLREFKNATK
GLADDDDDTKSTNVQKEKA**

TatC

**MTMMTPNQQTSKKKKRKGRKGRVPMQDMSIMDHAEELRRRIF
VVLAFFIVALIGGFFLAVPVITFLQNSPQAADMPFNAFRLTDPLRV
YMNFAVITALVLIIPVILYQLWAFVSPGLKENEQKATLAYIPIAFL
LFLAGIAFSYFILLPFVISFMGQMADRLEINEMYGINEYFSFLFQL
TIPFGLLFQLPVVVMFLTRLGVVTPPTFLRKIRKYAYFALLVIAGII
TPPELTSHLFVTVPMLILYEISITISAITYRKYHGTTDHNGQESAK**

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